Histamine Levels in Repeated Thawing Beef

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Abstract
The aim of this research was to determine the histamine levels in repeated thawing beef. This research were using longisimus dorsi part (sirloin) of beef. Repeated thawing treatments of beef consisted of P_1 as control without freezing, P_2 as first thawing after one week freezing, P_3 as second thawing without freezing two weeks and P_4 with freezing three weeks. The slices of beef was storage in freezer at temperature of -20ºC every weeks for three weeks. The thawing process was done by storage in refrigerator at temperature of 6ºC for 270 minutes after being removed from the storage in freezer every weeks for three weeks. PIC (Protease Inhibiting Cocktail) methode were used to extraction of enzyme in beef. Enzyme Link Imunosorbent Assay (ELISA) were used to detected the histamine levels with 450 nm wave length. The quantitative data from parameters were analyzed by descriptive. The results of the study concluded that repeated thawing beef in refrigerator at temperature of 6ºC could decrease the histamine levels.
Keywords : histamine, beef, thawing

Introduction

Meat is one of the types of foodstuffs of animal origin, including beef which can hardly be separated from human life (Nurwantoro *et al*., 2012). Beef has enough role in national food context because it is one commodity with a complete nutrient (Emhar *et al*., 2014). Chemical composition of beef consists of 56-72 % water , 15-22 % protein , 5- 34 % fat and 3.5 % protein not dissolved, include carbohydrates, organic salts, substances dissolved nitrogen, minerals and vitamins (Afiati, 2009) .

Beef has a lot of benefits as nutritional value, especially protein. Futhermore it could being lost if handled inappropriately because beef is an excellent medium for the growth of microbes, especially bacteria. The existence of bacteria in beef that often cause damage and can even lead to the source of the disease in humans. Bacterial contaminated beef trhroughout cattle life and since slaughtered, the process of preparing carcass until the beef will be consumed (Boediono *et al*., 2012) .

High demand for beef will increase at certain times, for example on religious holidays. The price of meat will increase certainly. It was caused by high demand but availability low for beef. People usually provide meat one day or two days before the celebration day, then stored in freezer (Maaniaan , 2009). Generally, upper and midle class of beef consumer, catering facilities or sellers of meat buy beef with cheap price and put it into freezer as soon as posible, then beef will be thawing before it was sold and cooked (Suryaningsih, 2010).

Beef storage by freezing can affect the conditions of microbial contamination. Some of bacteria contaminated beef can produce the enzyme L - Histidine Decarboxylase (HDC) which is a former precursor of the biogenic amines histamine. This compounds can produced by *Raoultella terrigena*, *Enterobacter* spp., *Microbacterium testaceum*, *Brevibacterium mcbrellneri, Diversus Micrococcus, Staphylococcus* spp (Heruwati *et al*., 2008).

Histamine is a biogenic amine compound produced from decarboxylation of free histidine (α-amine-β-propionic acid inidosal) (Lehane and Olley, 1999). This compound is an important mediator of immediate type allergic reactions (immediate). Allergic reactio by histamine has different effects based on receptor binding sites. In the skin, histamine causes dilation and permeability of capillaries which cause redness, burning sensation, itching and swelling. In the heart, histamine will increase heart rate. In the respiratory system, histamine causes bronchocontricsion (increase of bronchus contraction), so it is very significant in patients with bronchial asthma. In the digestive system histamine will increase gastric acid secretion and intestinal peristaltic which causes vomiting and diarrhea, other effects caused by histamine are increase secretion gland of saliva, pancreas, bronchus and tear but generally these effect are weak and not fixed (Syarif *et al*., 2011).

Histamine formation process was influenced by presence of L - Histidine Decarboxylase (HDC) (Bennour *et al*., 1991). Human body has effective system to detoxify toxins in the digestive tract. Monoamine oxidase, diamine oxidase and histamine N - methyltransferase are digestive enzyme which

metabolize histamine into the digestive tract become non-toxic compounds. However, possibility of histamine poisoning still need to warn because histamine detoxification system only works on condition of the daily normal intake (Indriati *et al.,* 2006). At very high intake of histamine, the system will not able to detoxify of this compounds. 15 ppm levels of histamine consumption can cause allergic symptoms and 100 ppm consumption may cause poisoning (Viciana *et al*., 1995)
Based on the results from the previous studies, the authors would like to explain the effect of repeated thawing in refrigerator to the levels of histamine in beef.

Materiale and methods

Procedure

Beef Freezing and Thawing

250 grams beef were divided into 4 treatments, a treatment consisted of six repetitions with different samples and each sample weight of 10 grams, the first treatment (P1) was not done freezing (preparated and extracted directly), others 3 treatments (P2, P3, P4) were frozen in a freezer at -20ºC temperature with thawing every week. Storage of beef (P2, P3 and P4) were done for one, two and three weeks, respectively. Thawing conducted at temperature of 6ºC in refrigerator until the entire ice melt for 270 minutes with a permanent packaging.

Preparation of Beef Extract

10 grams of thawed beef was washed with cold PBS once, then it cut into small pieces and taken 10 mg to incorporated into the micro tube, protease inhibitor 500µL added and RIFA buffer (1:100), cooled for 30 minutes in the freezer, then vortex using multi voltexer for 15 minutes, and centrifuged using a centrifuge cool on speed 10,000 rpm at 4°C for 20 minutes, then the supernatant was tested.

Histamine Assays Using ELISA Test

Some steps were used to test the levels of histamine in beef using ELISA techniques. 50 mL sample was inserted in the ELISA plate wells, then added 50 mL Biotinylated Detection Ab, incubated for 45 minutes at 37ºC temperature, then it was washed with ELISA washer for three times, then it was added 100 uL HRP Conjugate for each ELISA plate wells and incubated again for 30 minutes at 37ºC temperature, then it was washed with ELISA Washer five times, then added 90 µL Substrate Reagent pitting each ELISA plate and incubated for 15 minutes at a temperature of 37ºC, then added 50 uL Stop Solution and read its absorbance with ELISA reader at 450 nm wave length.

Data Analysis

Result datas will be analyzed by descriptive analysis.

Results and Discussion

The result of ELISA test of beef which repeated thawing was shown in Table 1 .

Table 1. Histamine levels (ppm) in beef after repeated thawing

Repetitions	No thawing	1st thawing (one week in freezer)	2nd thawing (two weeks in freezer)	3rd thawing (three weeks in freezer)
1	6.108×10^{-6}	4.312×10^{-6}	0.006×10^{-6}	0.002×10^{-6}
2	7.642×10^{-6}	9.514×10^{-6}	2.636×10^{-6}	0.686×10^{-6}
3	0.856×10^{-6}	0.456×10^{-6}	0.026×10^{-6}	4.144×10^{-6}
4	0.004×10^{-6}	0.204×10^{-6}	0.054×10^{-6}	1.046×10^{-6}
5	0.15×10^{-6}	0.048×10^{-6}	0.516×10^{-6}	0.292×10^{-6}
6	4.416×10^{-6}	16.66×10^{-6}	7.21×10^{-6}	1.208×10^{-6}
avarage	3.20×10^{-6}	5.20×10^{-6}	1.74×10^{-6}	1.23×10^{-6}

The results showed that the histamine levels lowest in the second week (P3) and then third week (P4) along duration of freezing and frequent thawing treatment, there were 1.74×10^{-6} ppm and 1.23×10^{-6} ppm (Table 1 and figure 1). Histamine levels increased during the first week of thawing treatment (P1) was 5.20×10^{-6}. it was may be caused by freezing storage at -20°C, which the HDC enzyme (L-Histidine Decarboxylase) inactivated to produce the histamine, so it could reduced or stopped.

According to Matulessy *et al*., (2010), the inhibition of cellular metabolism in the body tissues of animals to produce HDC enzyme will take place at -18°C temperature. HDC enzyme activity was as precursor of histamine formation influenced by pH, temperature and low oxygen availability, the highest enzyme activity at 37°C and the optimum pH was 6.0 (Eitenmiller *et al.,* 1982 ;Kusumawati and Indriati, 2008). Thawing process was done over -10°C temperature, bacterial activity will return

normally, although some of them died. Sublethal bacteria will also die over time in freezing storage. it caused increase the level of histamine in P_2. Maybe due to the presence of HDC enzyme as bacteria production. It was active again after sublethal phase for one week in freezing storage, then it decreased at P_3 and P_4 caused of continuous freezing treatment, then sublethal bacteria died and activity of HDC enzyme also halted because temperature was too extreme. Some of the HDC enzyme was produced by bacteria in sublethal phase at -20°C (Matulessye *et al*., 2010). The growth of bacteria, histamine forming and activity of HDC enzyme can be inhibited at 5°C or lower. Example Enterobacter, one of a family of Gram negative psychrotrophic.

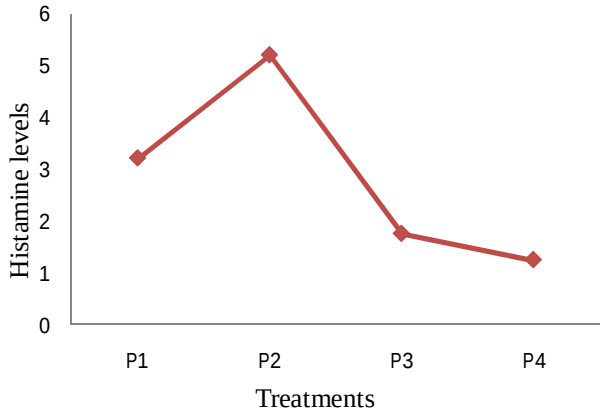

Figure 1. Graph histamine levels after tested ELISA

Beef freezing was done by freezing the beef below the froze point of the liquid at temperatur of -20ºC to -45ºC could inhibit the growth of microbes, proteolytic processes, hydrolysis processes, lipolytic processes and slight oxidative processes (Safitra and Putra, 2013). This statement was supported by Lawrie (1991), clotting speed will increase in line with the decline of temperature at -20°C. Almost 98% of the water contained in beef will freeze quickly and produce soft ice crystals, whereas the slow freezing will form large ice crystals could damage cells and caused denaturation of proteins. In such this stuations bacteria will grow faster in denaturated protein medium.

Possibility the lowest histamine levels in the second and third week of freezig storage not causing allergy and poisoning if consumed. Thawing treatment on meat at temperature of 6°C for 270 minutes in the refrigerator can reduce histamine levels. It could happen due to no microbial contamination in meat, so it safe for consumption. According to Suryaningsih (2010) beef thawing process is done by putting the frozen beef at temperature 5-10°C and let at least 1 hour to inhibit microbial recontamination.

Conclusion

Storage of beef in the freezer at temperature of -20 °C and repeated thawing in refrigerator at temperature of 6 °C could decrease the level of histamine.

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